

What is claimed is:

1. A method of preparing microsphere composite of collagen and bioceramic powder, comprising the steps of:

5 mixing a collagen solution with bioceramic powder and alginate to form a mixture solution;

squeezing the mixture solution to spherical droplet being discharged into a divalent cation solution undergoing gelling to produce microsphere;

coating the microsphere with a chitosan solution to support the shape of the microsphere; and

10 liquefying and washing out interior alginate and surface chitosan of the microsphere with an aqueous buffer solution, while collagen in the microsphere is reconstituted to fiber network at the same time to obtain microsphere composite.

2. The method as claimed in claim 1, wherein the microsphere composite contains about 1-99 % collagen by weight with respect to the total weight of the microsphere composite.

3. The method as claimed in claim 1, wherein the collagen solution is at a concentration of about 0.1 – 20 mg/ml.

4. The method as claimed in claim 1, wherein the bioceramic powder is selected from the group consisting of  $\alpha$ - or  $\beta$ -tricalcium phosphate, hydroxyapatite, calcium sulfate hemi-hydrate, calcium carbonate, and the mixture thereof.

5. The method as claimed in claim 1, wherein the microsphere composite contains about 1-99 % bioceramic powder by weight with respect to the total weight of the microsphere composite.

6. The method as claimed in claim 1, wherein the diameter of the bioceramic powder is about less than or equal to 5  $\mu$  m.

7. The method as claimed in claim 1, wherein the mixture solution contains about 0.1–10 w/v % alginate.
8. The method as claimed in claim 1, wherein the mixing step comprises the step of agitating the mixture solution well.
- 5 9. The method as claimed in claim 1, wherein the mixing step is carried out at about 0–10 °C.
- 10 10. The method as claimed in claim 1, wherein the squeezing step comprises the steps of transferring the mixture solution to a vessel having a needle or connecting with a needle, loading an air jet-syringe pump extrusion with the vessel, and operating the air jet-syringe pump extrusion to squeeze the mixture solution into spherical droplet.
11. The method as claimed in claim 1, wherein the divalent cation solution comprises an cation which is selected from group consisting of  $\text{Cu}^{2+}$ ,  $\text{Pb}^{2+}$ ,  $\text{Ca}^{2+}$ ,  $\text{Ba}^{2+}$ ,  $\text{Cd}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Ni}^{2+}$ , and  $\text{Sr}^{2+}$ .
- 15 12. The method as claimed in claim 1, wherein the divalent cation solution is at a concentration of about 0.1 – 10 w/v %.
13. The method as claimed in claim 1, wherein the chitosan solution is formed by dissolving chitosan in an acid solution, and the weight percentage of chitosan in the chitosan solution is about 0.00001 % - 10 %.
- 20 14. The method as claimed in claim 1, wherein the chitosan solution is formed by dissolving chitosan in an acid solution and the weight percentage of chitosan in the chitosan solution is about 0.00001 % - 10 %, said acid solution comprises an acid selected from the group consisting of formic acid, acetic acid, propionic acid, lactic acid, malic acid, citric acid, ascorbic acid, oxalic acid, succinic acid, malonic acid, adipic acid, pyruvic acid, glutaric acid, tartaric acid, asparagic acid, epoxysuccinic acid, monochloroacetic acid, salicylic acid, itaconic acid, pyrrolidone carboxylic
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acid, glycolic acid, nitric acid, sulfuric acid, hydrochloric acid, phosphoric acid, and the mixture thereof.

15. The method as claimed in claim 1, wherein the aqueous buffer solution is selected from the group consisting of phosphate buffer solution, sodium citrate solution, and the mixture thereof.

16. The method as claimed in claim 15, wherein the phosphate buffer solution is at a concentration of about 0.001 – 1M.

17. The method as claimed in claim 1, wherein the diameter of the microsphere composite is in the range of about 50  $\mu$ m – 5 mm.

18. The method as claimed in claim 1, wherein the liquefying is carried out at about 35-40 °C.